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# Appendix App

# Original Article Acute toxicity of Diazinon to the Caspian vimba, *Vimba vimba persa* (Cypriniformes: Cyprinidae)

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<b>Abstract:</b> The present research was performed to determine lethal concentrations of diazinon for the Caspian vimba, <i>Vimba vimba persa</i> . Fish samples $(50 \pm 5 \text{ g})$ collected from Sefidroud river were acclimatized for 5 days and exposed to 5 concentrations of diazinon, 0.07, 0.08, 0.1, 0.13 and 0.16 mg/l	Article history: Received 23 June 2013 Accepted 16 October 2013 Available online 25 December 2013
(with three replicates) and lethal concentrations (LC) LC1, LC10, LC30, LC50, LC70, LC90 and LC99 for 24, 48, 72 and 96 h were determined using a probit analysis. The results indicated that the 96 h LC50 value of diazinon for Caspian Vimba was 0.08 mg/l.	<i>Keywords:</i> Insecticide Diazinon Vimba

### Introduction

Organophosphate pesticides are a major group of chemical insecticides being used widely throughout the world (Garfitt et al., 2002) in agriculture, gardening, pest control, residential areas for protection of public health, veterinary and industry (Bonilla et al., 2008). These insecticides frequently enter into surface water and groundwater through drainage from agricultural fields (Sohrabi et al., 2001). Diazinon is one of the most frequently used organophosphate insecticides for control of insects in agriculture. It becomes degraded rapidly in the aquatic environment. Its half-life, in aerobic mineral soils, may be more than one month (Garfitt et al., 2002; Ahmadi et al., 2011). Although diazinon is rapidly degraded, it may remain biologically active in the soil up to six month and even more under certain conditions such as low temperature, low humidity, high alkalinity and lack of active microbial decomposers (Eisler, 1986). Diazinon enters into

aquatic ecosystem in large quantities and affects non-target organisms (Burkepile, 2000; Maxwell and Dutta, 2005).

 $LC_{50}$ 

Diazinon has been detected in significant amount in many coastal, deltaic and surface waters and also in municipal wastewater treatment plants around the world, including Iran (Shayeghi et al., 2001; U.S. EPA, 2005). It has been detected in some drainage of rice fields in north of Iran (Nouri et al., 2000; Tavakol, 2007) and Mahabad, Simine (Honarpajouh, 2003), Nahand (Tarahi Tabrizi, 2001), Kor, Sivand, Shahpoor, Mand, Dalki (Shayeghi et al., 2007), Gorgan, Gharesoo (Bagheri, 2007), Karaj river and most of Mazandaran rivers (Shayeghi et al., 2007; Arjmandi et al., 2010). Once entered into surface waters, even low doses of diazinon can cause several adverse effects in fish such as neurological impairment and abnormalities in gills, impairment in immune system, olfactory system and reproductive behavior, damage to ovary and testis and delay in

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Concentration (mg/l) 48 h 24 h 72 h 96 h Points  $LC_1$ 0.01 0.06 0.04 0.007 0.1  $LC_{10}$ 0.16 0.07 0.04  $LC_{30}$ 0.28 0.12 0/09 0.06 LC<sub>50</sub> 0.36 0.14 0.1 0.08 LC<sub>70</sub> 0.44 0.16 0.12 0.1  $LC_{90}$ 0.55 0.19 0.14 0.12 LC<sub>99</sub> 0.71 0.23 0.17 0.16

Table 1. Lethal concentrations of Diazinon for Caspian vimba.

sexual maturity (Eisler, 1986; Moore and Waring, 1996; Dutta et al., 1997, Dutta and Maxwell, 2003; Dutta and Meijer, 2003, Dutta et al., 2006). LC<sub>50</sub> of diazinon is highly variable, and depends on the age, weight, and genus of fish and the environmental conditions. It affects sexual hormones, increases LH and FSH level and decreases testosterone levels significantly in mice (Fattahi et al., 2009).

*Vimba vimba* is valuable species of cyprinid family (Schweyer et al., 1991) and lives in the Caspian Sea, Black Sea and eastern part of the North Sea. In Iran, Caspian Vimba is distributed in the southern Caspian Sea from the Anzali lagoon to the Gorgan River. Based on IUCN classification the Caspian Vimba (*Vimba vimba persa*) is a threatened species in the Caspian sea (Kiabi et al., 1999) and today the fish needs protection because of significant decline in stock, over fishing and destruction of habitat (Jolodar and Abdoli, 2004). The purpose of this research was to determine acute toxicity of diazinon to *Vimba vimba persa*.

## Materials and methods

Experiments were performed according to the OECD standard method (OECD, 1989), to determine 96 h LC<sub>50</sub> of diazinon to Caspian Vimba. For these experiments 90 fish with an average weight of 50  $\pm$  5 g were collected from Sefidroud river and were acclimatized for 5 days, then divided into five treatments and one control (with three replications) in same aquaria (30 × 40 × 70 cm). Physicochemical properties of water used for these experiments were as follows: 23  $\pm$  1°C temperature, 7 to 9.5 mg/L

dissolved oxygen, 6.5 to 8 pH and 220 mg/l total hardness. During the experiment, water was not exchanged. Before the test, fish were fed twice daily with Biomar feed at 2% body weight. Five concentrations of diazinon used were 0.07, 0.08, 0.1, 0.13 and 0.16 mg/l. The nominal concentration of diazinon causing 50% mortality of Caspian Vimba within 24 h (24 h LC<sub>50</sub>), 48 h, 72 h and 96 h was determined using probit analysis in the software SPSS 16.

#### Results

Values of different lethal concentrations of diazinon for 24-96 h to Caspian Vimba have been presented in Table 1. The results show that 96h LC<sub>50</sub> value of diazinon for Caspian Vimba is 0.08 mg/l.

#### Discussion

Contrasting results are available on acute toxicity of diazinon to fish. Tumer (2002) observed 96 h LC<sub>50</sub> values for some freshwater teleost greater than 90 mg/l. On the contrary 96 h LC<sub>50</sub> values of diazinon for *Acipenser persicus*, *Acipenser stellatus* and *Acipenser nudiventris* were determined as 4.38, 2.54 and 0.36 mg/l, respectively (Pazhand, 1999; Shamooshaki, 2005) and those for *Rutilus frisii kutum*, *Hypophthalmichthys molitrix* and *Abramis brama* were determined respectively as 0.34, 1.9 and 1.8 mg/l (Nasri Tajan, 1997). Compared to these researches, *Vimba vimba* is considered as highly sensitive and vulnerable to diazinon. 96 h LC<sub>50</sub> value of diazinon to Caspian vimba is even much lower than the sub lethal levels of diazinon (0.3 to 3.2

mg/L) that reduce emergence of stream insects cause potential mutagenicity in freshwater fish and spinal deformities in fish (Eisler, 1986).

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